

Best Available Copy

Serial No. 10/038,984
Group No. 1635
Confirmation No. 9705

⑪  **Europäisches Patentamt**
European Patent Office
Office européen des brevets

⑪ Publication number:

0 213 921
B1

⑫

EUROPEAN PATENT SPECIFICATION

④⑤ Date of publication of patent specification: 08.08.90

⑦① Application number: 86306582.7

⑦② Date of filing: 26.08.86

⑤① Int. Cl.⁵: **A 61 K 37/66, A 61 K 35/14 //**
(A61K37/66, 35:14, 31:70)

⑤④ **Modulation of virus-related events by double-stranded RNAs.**

③① Priority: 26.08.85 US 769494
17.07.86 US 886363

④④ Date of publication of application:
11.03.87 Bulletin 87/11

④⑤ Publication of the grant of the patent:
08.08.90 Bulletin 90/32

④④ Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

⑤⑥ References cited:
EP-A-0 113 162
GB-A-1 412 591

⑦③ Proprietor: **HEM RESEARCH, INC.**
12280 Wilkins Avenue P.O. Box 2245
Rockville, MD 20852 (US)

⑦② Inventor: **Carter, William A.**
1 Jaine Lane
Birchrunville Pennsylvania 19421 (US)

⑦④ Representative: **Smart, Peter John et al**
W.H. BECK, GREENER & CO 7 Stone Buildings
Lincoln's Inn
London WC2A 3SZ (GB)

EP 0 213 921 B1

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European patent convention).

Courier Press, Leamington Spa, England.

Description

AIDS (Acquired Immunodeficiency Disease Syndrome) represents a major public health threat of the Twentieth Century. Because of its biologically variable nature, its subtle means of spread from individual to individual, and its "latent" disease period which defies even the ready detection of its very presence, AIDS has rapidly evolved into an epidemiologic dilemma of unprecedented proportions. Indeed, even with the considerable resources already deployed to increase diagnostic capabilities, the available technologies still fall well short of the necessary requirements to gain firm control of this problem within any section of the population (e.g., see Proceedings of Workshop entitled "Experience with HTLV-III Antibody Testing, an Update on Screening, Laboratory and Epidemiological Correlations", sponsored by the Center for Drugs and Biologics, FDA, National Institutes of Health, and Centers for Disease Control, held on July 31, 1985, at the National Institute of Health, Bethesda, Maryland; see also, Budiansky, S. in *Nature*, volume 316, page 96, July 11, 1985). Two different designations for the AIDS virus exist; LAV is the designator for the AIDS virus isolated at the Pasteur Institute, Paris, France and HTLV-III is the designator for the AIDS virus isolated at the National Institute of Health, Bethesda, Maryland, U.S.A. Frequently in this text, the AIDS virus will be referred to generically or designated HTLV III or LAV without intending to differentiate between them. Furthermore, the term "AIDS virus" in the specification includes any and all other viruses which may be associated with producing AIDS, whether yet isolated or not.

Current therapeutic considerations have taken one of two approaches: immunological (vaccine production) or direct attacks on the virus itself (antiviral therapy). While vaccine production initially appeared extremely promising, at least in theory, new knowledge of the viral composition has significantly undermined this promise; namely, the virus apparently readily mutates or changes its basic biochemical structure such that critical viral antigenic "determinants"—necessary for vaccines to be effective—readily undergo mutation or change. For example, HTLV—III isolates in California, Maryland and England have recently been found to be significantly different from each other when their genomic content(s) has been rigorously analyzed. The implication of such studies is that vaccination against AIDS will not have widespread lasting benefits such as the conclusively beneficial/durable results historically characteristic of most other vaccines, such as those directed against polio virus, measles virus, etc.

In the second, or direct antiviral approach, other scientists have tested several compounds including HPA-23, suramin, ribavirin, interferon and foscarnet. These compounds have been introduced into either the laboratory or clinical studies with little-to-no evidence of therapeutic success to date. Indeed, consistent evidence of high toxicity and/or severe side-effects has been reported in nearly every instance (e.g., see summary prepared by M. Clark, *Newsweek*, page 71, August 5, 1985; also, C. Wallis, *Time*, pages 40—47, August 12, 1985). None of these drugs are indeed new or able to be selectively directed against the underlying disorder; namely, multiplication of AIDS virus in certain cells. For example, HPA-23 is a combination of heavy metals (reminiscent of the use of arsenic to treat venereal diseases in the 1920s, or pre-penicillin era); suramin is actually an anti-parasitic (sleeping sickness) compound and foscarnet is an anti-herpes virus compound. The latter two compounds may inhibit an AIDS-virus related enzyme termed "reverse transcriptase", but there is no evidence that such an enzymatic inhibition, in fact, would result in any therapeutic improvement or disease prevention/amelioration. Similarly, interferon, which is as toxic and non-specific as the above-mentioned compounds, may have some limited activity on AIDS-virus related tumors, but numerous studies indicate that it holds little promise as an effective antiviral against AIDS virus, or most other viruses for that matter.

Mismatched double-stranded RNA is a known form of macromolecular RNA (see U.S. Patent No. 4024222 and U.S. Patent No. 4130641) in which destabilization of the double helix prevents base pairing. Mismatched dsRNA is well known for its interferon-induction properties which indicate a mechanism unrelated to interferon induction *per se* (e.g., see European Patent Application EP—A—113162 filed August 15, 1983 entitled "Antiproliferative Action of dsRNAs on Tumor Cells"). It is known from GB—A—1412591 that dsRNAs exhibit a certain antiviral activity. A typical therapeutic embodiment of mismatched double-stranded RNA is the synthetic dsRNA formed by complexes of polyriboinosinic and polyribocytidylic/uridylic acid, such as $rI_n \cdot r(C_xU \text{ or } G)_n$, where x has a value from 4 to 29, e.g. $rI_n \cdot r(C_{12}U)_n$, herein referred to as "Ampligen", a trademark of HEM Research, Inc., of Rockville, Maryland, USA. Many mismatched dsRNA polymers which behave similarly to Ampligen have been studied. The key therapeutic advantage of mismatched dsRNAs over other forms of natural and/or synthetic dsRNAs is their reduced toxicity in animals and man. For example, Lampson et al in U.S. Patent No. 3666646 described earlier complexes of dsRNA which are capable of triggering various interferon-related effects, but the toxicity of such compounds precluded any clinical utility in the treatment of cancer or related disorders.

The inventor has fully described the known activities of various interferons, interferon inducers and dsRNAs in his recent textbooks on the subject (e.g., see the inventor's chapter in *Anticancer and Interferon Agents*, pages 301 through 319, edited by R. M. Ottenbrite and G. B. Butler, Marcel Dekker, New York, 1984; also see *Handbook of Experimental Pharmacology on Interferons*, edited by P. E. Come and W. A. Carter, 1984, published by Springer-Verlag, New York and Heidelberg; pages 535—555 fully describe the known properties of dsRNAs).

Mismatched dsRNA, e.g., Ampligen, is now known to have therapeutic activity against certain human tumors, particularly kidney cancer. Although currently thought of as purely an "interferon inducer",

dsRNAs are active on certain human tumors which are completely resistant to interferon itself as fully described in European Patent Application 83305426.5 of which the present applicant is an inventor.

Similar to the lack of activity against certain tumors, it has now been demonstrated that virtually all types of interferon exhibit no significant activity in the treatment of the AIDS virus. Being known "interferon inducers", it is particularly surprising that mismatched dsRNAs have application in the treatment of AIDS-related disorders.

The present invention is based on another new and unexpected property of dsRNA, especially mismatched dsRNA, as exemplified by Ampligen, which has a surprisingly wide range of applicability to the treatment of AIDS-related disorders. The invention encompasses the use of these materials to treat against retroviruses and T-cell lymphotropic viruses (whether retroviruses or not) generally.

The present invention provides the use of a dsRNA in the manufacture of a composition for use in the treatment of retrovirus or T-cell lymphotropic virus induced conditions, e.g. AIDS, by therapy or prophylaxis.

An important symptom of AIDS is the suppression of the normal immune state as indicated by the immune skin response. This is restored during successful treatment with dsRNA.

Accordingly, the invention includes the use of dsRNA in the manufacture of a medicament for restoring an anergic immune state to a substantially normal immune state as measured by testing the immune skin response.

The dsRNA may be a mismatched dsRNA.

HTLV III/LAV infection produces a particularly wide range of severity of response from relatively asymptomatic carriers to profound immunological paralysis. To treat this range of response, a wide range of dosage of dsRNA may be appropriate. When low dosages are employed, the use of non-mismatched dsRNA may be appropriate and dosages may be sufficiently low to avoid the adverse reactions mentioned above. Where higher doses are appropriate the need to use mismatched dsRNA will be greater.

Preferably, the dsRNA is such as to produce an elevated intracellular concentration of 2'—5'A oligomers without host toxicity, e.g. due to an accelerated bioavailability, a structure adapted to activate 2'—5'A oligomer production without producing side effects, or due to a relatively short half life following administration.

By "matched dsRNA" are meant those in which hydrogen bonding (base stacking) between the counterpart strands is relatively intact, i.e. is interrupted on average less than one base pair in every 29 consecutive base residues. The term "mis-matched dsRNA" should be understood accordingly.

The dsRNA may be a complex of a polyinosinate and a polycytidylylate containing a proportion of uracil bases or guanidine bases, e.g. from 1 in 5 to 1 in 30 such bases (poly I. poly (C4—29x>U or G).

The dsRNA may be of the general formula $rI_n \cdot (C_{12}U)_n$. Other suitable examples of dsRNA are discussed below.

The composition may comprise the dsRNA and an AIDS inhibition assisting agent, such as a lymphokine, e.g. an interferon or an interleukin such as IL-2.

The invention accordingly includes the use of dsRNA in the manufacture of a pharmaceutical composition for the prevention or treatment of AIDS in a person comprising, in combination, a dsRNA and a lymphokine such as an interferon.

Preferably the composition contains the dsRNA and the interferon in the ratio of 0.01 to 1000 micrograms of dsRNA to 0.1 to 100,000 IRU of interferon.

The invention further includes a method of rendering a, preferably human-originating, biological fluid or, preferably human-originating, cells resistant to viral infection, enhancing the resistance thereof, or mitigating the effects of infection therefrom comprising admixing or contacting said biological fluid or cells with an effective, e.g. AIDS-inhibiting, amount of a dsRNA.

The biological fluid may be human blood or a fraction thereof for instance for use in transfusion or dialysis.

The invention further includes as a composition of matter, human blood or a fraction thereof or human cells and an effective, e.g. AIDS-inhibiting, amount of a mismatched dsRNA.

Furthermore, the invention includes also a process of removing or inactivating AIDS viruses from a device for handling parenteral fluids or mitigating the effect of infection therefrom comprising contacting said device with a composition containing an effective, e.g. AIDS-inhibiting, amount of a dsRNA.

The invention includes also a composition for use in the treatment of AIDS comprising a dsRNA and a pharmaceutically acceptable carrier or diluent together with instructions for use in the treatment of retroviruses or T-cell lymphotropic virus induced conditions, e.g. AIDS.

Thus there are disclosed hereinafter by way of example, therapeutic methods of selectively inhibiting human viruses, specifically the AIDS virus, without significant toxicity to normal cells; methods of inhibiting, delaying or preventing a person from becoming infected with AIDS virus; methods of preventing or treating AIDS-related disorders in humans; methods of treating human biological fluids, cells and tissue products to prevent or arrest infection or contamination with AIDS virus; and methods of correcting specific lesions associated with AIDS virus, including loss of interferon receptors on cells within the immune system, curtailment or reduction of intracellular 2'—5'A synthetase and the reduction or loss of intracellular dsRNAs in selected cells of the immune system. Pharmaceutical compositions for use in these methods are also disclosed.

The use of dsRNA is adapted to correct specific lesions associated with AIDS virus, including loss of interferon receptors on various critical cells within the immune system, curtailment/reduction of intracellular 2'—5'A synthetase in various critical cells within the immune/bodily defense system, and reduction or loss of intracellular dsRNA in various critical cells within the immune/bodily defense system.

5 The mismatched dsRNAs preferred for use in the present invention are based on copolynucleotides selected from poly (C_n,U) and poly (C_n,G) in which n is an integer having a value of from 4 to 29 and are mismatched analogs of complexes of polyriboinosinic and polyribocytidilic acids, formed by modifying rI_n · rC_n to incorporate unpaired bases (uracyl or guanine) along the polyribocytidylate (rC_n) strand. Alternatively, the dsRNA may be derived from poly (I), poly (C) dsRNA by modifying the ribosyl backbone of polyriboinosinic acid (rI_n) e.g. by including 2'-O-methyl ribosyl residues. These mismatched analogs of rI_n · rC_n, preferred ones of which are of the general formula rI_n · r(C₁₂,U)_n, are described by Carter and Ts'o in U.S. Patents 4,130,641 and 4,024,222. The dsRNA's described therein generally are suitable for use according to the present invention.

Other examples of mismatched dsRNA for use in the invention include:—

15 poly (I) · poly (C₄, U)
poly (I) · poly (C₇, U)
poly (I) · poly (C₁₃, U)
poly (I) · poly (C₂₂, U)
poly (I) · poly (C₂₀, G)
20 poly (I) · poly (C₂₉, G) and
poly (I) · poly (C_p) 23 G>p.

Pharmaceutical compositions in accordance with this invention include the mismatched dsRNA, optionally also an interferon, together with a pharmaceutically acceptable carrier or diluent. Pharmaceutical compositions contemplated by this invention include those adapted for parenteral administration in a suitable pharmaceutical vehicle.

Thus, for example, parenteral solutions, suspensions and dispersions can be prepared as required according to known pharmaceutical techniques with sterile or pyrogen-free water as the solvent/diluent optionally also with physiologically acceptable salts.

It will be understood that the absolute quantity of active ingredients present in any dosage unit should 30 not exceed that appropriate to the rate and manner of administration to be employed, but on the other hand should also desirably be adequate to allow the desired rate of administration to be achieved by a small number of doses. The rate of administration will moreover depend on the precise pharmacological action desired.

The amount of mismatched dsRNA administered is preferably sufficient to achieve a level of from 0.01 35 micrograms per millilitre of body fluid after equilibration of the dsRNA level through the body fluid up to 1000 micrograms per milliliter in the systemic circulation immediately following administration. When concurrently administered, interferon is preferably included in an amount that results in a level of 0.1 to 100,000 IRU per milliliter of body fluid. Generally, the amount to be administered will depend on the severity of the condition, in particular the level of available intracellular bioactive dsRNA, 2'—5' oligo A 40 molecules or 2'—5'A synthetase. As used herein, the term body fluid is the solution of serum, vitamins, etc. which circulates within the organism and bathes the tissues. Expected body fluid volumes of patients are of course known to practitioners and are published as charts and tables, which are routinely available.

As indicated above, the invention includes the treatment of biological fluids with dsRNA and similarly the treatment of cells with dsRNA. Blood products such as whole blood used in transfusions or 45 components of whole blood, such as "packed (concentrated) red blood cells, packed white blood cells, platelet concentrates or serum protein fractions such as immunoglobulins" may be treated in this way. Such blood products may have mismatched dsRNA at appropriate concentrations added to them at the time of initial isolation and prior to any cryopreservation.

Alternatively, an effective concentration may be added to the blood product immediately prior to 50 injection into the recipient. In such cases, the operator may simply refer to a standard table of body fluid volumes which interrelate the weight of the recipient to his or her body fluid volume, which is the total of the body fluid volume of the patient and the body fluid volume available for equilibration with the necessary quantity of the dsRNA. A sixty to seventy kilogram patient will have a body fluid volume of approximately five to six litres.

55 Blood products as described above may have undetected retroviruses, especially AIDS-related virus associated with them. The objective is to provide a final concentration in the blood product in question which prevents the "seeding" of the occult retroviruses in the diverse tissues of the recipient of the blood product. By preventing a successful seeding of such retrovirus one can prevent what otherwise would become a life threatening viral proliferation when the contaminated transfused cells and/or cell products are admixed and/or distributed throughout the recipient's system.

60 Other important uses for this technique are instances where the patient's blood is transitorily exposed to donor blood and/or blood products, such as through a membrane which may be defective or may serve to transmit retroviruses, examples of such instances include the use of equipment such as extracorporeal pumps and associated devices used during cardiac surgery, cardiac bypass surgery and organ transplants. 65 This differs from the situation in which whole blood or blood products are infused into the patient.

EP 0 213 921 B1

Transitory exposure through a membrane involves only temporary exposure to possibly contaminated blood, exposure lasting as little as a few minutes or for as long as several hours depending on the length of the surgical or other medical procedure. Similar exposure is possible with renal dialysis equipment in which the presence of an effective concentration of dsRNA will thwart the possibility that undetected retroviruses may traverse membranes and seed the body of an individual connected to such equipment.

Medical literature also documents the passage of the AIDS-related virus through nasal and lachrymal secretion. Medical equipment and devices having the potential to transfer retroviruses from one patient to another are also effectively treated with mismatched dsRNAs according to the invention. Illustrative examples include inhalation and inhalation therapy equipment used to assist in respiration as well as instruments used to examine and treat eyes.

Aerosols containing dsRNAs may be sprayed onto the contacted portions of the device to prevent contamination of equipment due to multiple users.

The amount of dsRNA included in or added to whole blood or blood products will depend on the overall dilution rate of the blood or blood products in use. First, the tissue volume of the patient may be determined based upon the size of the patient, then the quantity of dsRNA may be calculated. A transferred blood product will require a greater concentration of the dsRNA because of the extensive overall dilution as compared with an extracorporeal blood pump or dialysis equipment using a membrane in which the donor patient is to be protected without concern for dilution to immobilise any retrovirus in the donated material. Preferred amounts for non-diluted uses are in the range of from 0.1 microgram to 200 micrograms per millilitre of body fluid. In contrast, for whole blood, usually available in containers or 350 and 500 ml, a sixty kilogram patient with a whole body fluid volume of between about five and six litres will require about 200 mg of dsRNA to achieve a concentration of 40 micrograms per millilitre upon equilibration.

The invention will be illustrated by the following description of examples of the use of dsRNA.

Mismatched dsRNAs, e.g., Ampligen, were formulated in aqueous solution such that, when added to various human cells, transient concentrations of 0.01 microgram to 250 microgram per milliliter of fluid (bathing cells) was obtained. A variety of different cells, all potential targets for infection with HTLV-III (AIDS virus), were used.

Below described is a typical experiment with H-9 cells, a human lymphoid cell which can be acutely or chronically infected with HTLV. The cells were grown under standard conditions (e.g., see Mitsuya et al, *Science* volume 226, page 172, 1984) and analyzed for several weeks with respect to the presence of HTLV enzymes or HTLV-specific proteins. Various concentrations of Ampligen were added, before, after or simultaneously with the virus to thus mimic various clinical conditions. Most importantly, careful analyses of cell number and morphology, were done to determine if the mismatched dsRNA had various non specific effects on lymphoid cell growth as had been described by Mitsuya with other inhibitors. Cells, at different times during the experiment, were isolated and studied by HPLC analysis of the 2',5'-A oligomers using methods described by Lee et al (*Biochemistry*, volume 24, page 551, 1985). Certain A oligomers, when present in nature, are known to confer viral resistance to cells, but it has not been previously described, or anticipated, that a test compound (synthesized by man) could precisely trigger this natural reaction selectively, and thus strengthen a natural defense mechanism against viruses in general, and AIDS virus in particular.

TABLE 1
Effect of ampligen on HTLV-III infectivity

Experiment A					
Day	%HTLV positive cells		Reverse transcriptase		
	+ Drug	- Drug	+ Drug	- Drug	
4	0	50	1,412	24,287	
9	7	90	144,632	1,243,300	
Experiment B					
3	0	0	353	323	
7	0	0	400	1,200	
10	0	5	800	2,261	
14	2	>80	1,100	112,247	
17	5	>90	1,200	1,560,000	

EP 0 213 921 B1

Experiment A was conducted with 25 times more virus (expressed as infectious units) than target cells whereas Experiment B was conducted with 10 times more cells (designated H-9 cells) than virus. Percent cells positive refers to cells which expressed HTLV-III markers designated p24 and p19 as determined by immunofluorescence; reverse transcriptase refers to a viral enzyme measured in the cell supernatant by the standard template designated poly rA/dT. Concurrently, cell counts were done: the cells multiplied at normal growth rates at all concentrations of Ampligen tested (up to 300 micrograms per milliliter). In experiments A and B, Ampligen (50 micrograms per milliliter) was added on day 1. HPLC analysis showed (by day 3-7 in either Experiment A or B) a specific shift in the 2',5'-oligomer profile such that the higher (less antiviral) molecular weight (MW) oligomers shifted to the lower (most antiviral) MW oligomers which contributed to a selective and strong suppression of the AIDS virus.

Similar experiments were conducted with various other human cells which are potential *in vivo* targets for AIDS virus: These experiments included other cells functionally determined to be NK (natural killer) cells, T helper cells, T suppressor cells and mononuclear cells, etc. In all instances, various concentrations of mismatched dsRNA were able to selectively arrest and/or prevent HTLV multiplication without any effect on normal cell growth and maturation. Proliferation of the T cells in culture was maintained by the standard addition of IL-2 factor as well known to those familiar in the art of cell biology. The selective suppression of HTLV by mismatched dsRNA was consistently associated with a specific shift, or enhancement, in the 2',5'-oligomer profile as determined by HPLC analysis.

Comparisons of mismatched dsRNAs with interferon

Since dsRNAs, being "interferon inducers", might be viewed as simply working through (via) an interferon mechanism, any activity of interferon *per se* might be thought to suggest activity by dsRNA. Thus, the following experiments establish that uniqueness of the present invention by demonstrating no significant activity of interferons of virtually all types in the face of very potent and specific anti-AIDS virus activity by Ampligen.

CEM (another human T cell line) cells were treated with either 250 I.U./ml recombinant alpha interferon, 250 I.U./ml natural beta interferon, 50 I.U./ml natural gamma interferon or 50 µg/ml Ampligen for 18 hours prior to infection with LAV. After 15 days of culture, the following data were collected.

TABLE 2
Effect of ampligen on LAV infectivity

	Reverse transcriptase [cpm/ml culture fluid]	indirect immunofluorescence [% of cells with LAV antibody binding]
untreated	1,147,000	49%
alpha interferon	503,000	5%
beta interferon	1,500,000	60%
gamma interferon	360,000	30%
Ampligen	24,000	<1%

The data indicate a profound inhibition of viral replication by Ampligen.

Thus, dsRNA (Ampligen) inhibits (>99%) HTLV-III/LAV infection in H9 (T4) cells and CEM (T4) cells. Interferons alpha, beta and gamma are marginally active or inactive. Viral inhibition by Ampligen is very selective and occurs without any measurable effect on cell growth.

Thus, Ampligen inhibits HTLV-III/LAV virus replication by a mechanism distinct from interferon. This finding suggests that Ampligen and interferon or other drugs could be used in combination, whereby Ampligen would inhibit viral replication and the interferon could be used in stimulating the immune system to restore pre-disease function.

In the restoration of the natural antiviral state Ampligen acts distal to the AIDS/ARC block in the antiviral pathway to restore a natural antiviral RNase activity which destroys the viral genome.

Therefore, the drug has the potential to inhibit viral replication, as well as to augment the immune response. Thus, it should be noted that Ampligen is not simply a reverse transcriptase inhibitor of the virus, but acts through the dual mechanisms of human immune system stimulation and establishment of an antiviral state in target cells. Ampligen is readily set apart from interferons, in that it may well be able to cure viral infections and viral induced tumors in animals which are completely refractory to exogenous interferon therapy. These observations distinguish Ampligen from many other drugs proposed for use against this disease.

In related studies the pathophysiology of AIDS was studied and unexpected and previously undetected biochemical lesions in individuals predisposed (homosexual) to AIDS were detected. Such lesions are

EP 0 213 921 B1

correctable by Ampligen and explain its potent antiviral activity in the face of nominal activity (if any), by interferons or most other compounds which have been tested.

First, it was observed that the missing critical biochemical co-factor was dsRNA, in the T lymphocytes of each of 6 individuals predisposed to AIDS (homosexual males) and in each of 6 AIDS victims. Measurements were made of dsRNAs by standard techniques following cell disruption. The effect of Ampligen can thus be accurately viewed as *specific replacement therapy* for a critical biochemical entity needed for humans to withstand virus attack at the subcellular level. When dsRNA is present naturally or added, as via Ampligen therapy, an active RNase L is formed which then "eats up" (hydrolyzes), or destroys, the viral mRNA thus stopping or containing the viral infection. Subsequently to the filing of the USA priority application, Preeble et al reached a complementary conclusion (September 1985 issue of *Journal of Infectious Diseases*, pages 457-465, entitled "Interferon Induced 2'-5' Oligoadenylate Synthetase During Interferon Alpha Therapy in Homosexual Men with Kaposi's Sarcoma: Marked Deficiency in Biochemical Response to Interferon in Patients with Acquired Immunodeficiency Syndrome"). They showed that the addition of interferon to immune cells from AIDS victims did not result in the expected boost in this specific intracellular pathway, a first line of defense against various viruses.

Second, it was determined that AIDS virus infection itself caused a further loss, or alteration, in the interferon receptor on the cell surface such that interferon could not bind well and could not therefore initiate the critical "cascade" of biochemical events normally leading to arrest of viral infection and restoration/maintenance of immune competence. It was further observed that unusually low levels of 2'-5' A synthetase follow HTLV-III infection. Thus, AIDS virus infection itself is characterized by applicant at least three (3) deleterious events which however, can be specifically remedied by Ampligen or dsRNA therapy: (1) loss of interferon receptors, (2) reduction in 2'-5' A synthetase, and (3) abnormally low intracellular dsRNA.

In the relevant biochemical pathway, *dsRNA works downstream or distal to the biochemical lesions in individuals at risk* (with or without active AIDS virus infection), thus restoring normal antiviral response capability.

dsRNA is very efficiently taken up by cells and therefore does not require an intact IFN receptor. Further, mismatched dsRNA is also available as bioactive fragments to accelerate intracellular uptake and distribution. Thus, dsRNA has the potential to readily cross the blood-brain barrier, which may be a residual reservoir of AIDS virus in some individuals.

Typically, dsRNA for use in the present invention will be of the molecular size exemplified in U.S. Patents 4024222 and 4130641 but much lower molecular weight dsRNA, obtainable as fragments of the parent molecule are also effective. These may for instance be of one half to one tenth the size of the typical dsRNA materials described previously.

dsRNA activates the 2'-5' A polymerases and promotes the synthesis of a 500-fold increment in the active antiviral oligonucleotides over that possible with interferons alone (dsRNA leads to 250 nanomoles of 2'-5' oligo A in 1.6×10^6 cells whereas 200 units/ml IFN lead to only 0.5 nanomoles of 2'-5' oligo A).

Similar phenomena were observed by applicant in the NK cells of individuals at risk to development of AIDS, or possessing frank AIDS. A similar situation exists with respect to the levels of Natural Killer cell (NK) activity in AIDS/ARC patients. However, much less is known regarding the mechanisms of NK regulation.

AIDS/ARC patients and healthy members of "at-risk" groups often have weak immunosurveillance capacity (functional NK and T lymphocytes) and cannot be re-activated by interferons. Ampligen acts distal to the disease block and can activate cytotoxic lymphocytes.

The above theories have been tested clinically in the following confidential trial.

A 60 kg adult male with an AIDS-related complex of intermediate severity (lymph nodes enlarged and unable to eat solid foods for almost one year due to enlarged lymph nodes, AIDS-virus concentration greater than 10^5 of particles per ml of blood but having no evidence of tumors or other infections) was treated with 200 mg mismatched dsRNA (Ampligen) admixed with physiologic saline solution by intravenous drip over a period of 30 minutes. This infusion resulted in a concentration of approximately 40 mcg (0.04 mg) per ml of body fluid when the dsRNA was completely circulated throughout the body and fully equilibrated with all of the extracellular body fluid. The dsRNA was infused into the patient on 6 consecutive treatment intervals spaced 2-3 days apart and was sufficient to rid the patient's body of all measurable AIDS-related virus and to correct the patient's deteriorated immune function. Restoration of the immune function was indicated by a 50% improvement in the ratio of T4/T8 lymphocytes, a ratio recognized as a reliable measurement of general immune capability with respect to retroviruses. Further, at the conclusion of therapy the patient's immune capability, as measured by skin testing, returned to normal from the anergic condition that was measured prior to administration of the mismatched dsRNA. The patient was able to resume eating solid food at the conclusion of therapy and his condition continues to progress.

Claims for the Contracting States: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. The use of a dsRNA in the manufacture of a composition for use in the treatment of retrovirus or T-cell lymphotropic virus induced conditions, e.g. AIDS, by therapy or prophylaxis.

2. Use of a dsRNA as claimed in Claim 1 wherein the dsRNA is a mismatched dsRNA.

EP 0 213 921 B1

3. Use of a dsRNA as claimed in Claim 2 wherein the dsRNA is a complex of a polyinosinate and a polycytidylate containing a proportion of uracil or guanine bases e.g. from 1 in 5 to 1 in 30 such bases.
4. Use of a dsRNA as claimed in Claim 3, wherein the dsRNA is of the general formula $rl_n \cdot (C_{12}U)_n$.
5. Use of a dsRNA as claimed in any preceding claim, wherein the composition comprises the dsRNA and an AIDS inhibition assisting agent.
6. Use of a dsRNA as claimed in Claim 5, wherein said assisting agent is a lymphokine.
7. Use of a dsRNA as claimed in Claim 6, wherein the lymphokine is an interferon or an interleukin.
8. The use of dsRNA as claimed in Claim 7, wherein the composition contains the dsRNA and an interferon in the ratio of 0.01 to 1000 micrograms of dsRNA to 0.1 to 100,000 IRU of interferon.
9. A method of rendering a biological fluid or cells resistant to infection with retrovirus or T-cell lymphotropic virus, e.g. AIDS virus, enhancing the resistance thereof, or mitigating the effect of infection therefrom comprising admixing or contacting said biological fluid or cells with an effective amount of a dsRNA as defined in any one of Claims 1 to 4.
10. A method as claimed in Claim 9 in which the biological fluid is human blood or a fraction thereof, or the cells are NK cells, T-cells, T-suppressor cells, T-helper cells, spermatozoa, ova or fetal cells.
11. Product comprising human blood or a fraction thereof or human cells and an effective amount of a mismatched dsRNA as a medicament.
12. A process of removing or inactivating retroviruses or T-cell lymphotropic viruses, e.g. AIDS viruses from a device for handling parenteral fluids or mitigating the effect of infection therefrom comprising contacting said device with a composition containing an effective amount of a dsRNA.
13. The use of dsRNA in the manufacture of a medicament for restoring an anergic immune state to a substantially normal immune state, as measured by testing the immune skin response.

Claims for the Contracting State: AT

1. The use of a dsRNA in the manufacture of a composition for use in the treatment of retrovirus or T-cell lymphotropic virus induced conditions, e.g. AIDS, by therapy or prophylaxis.
2. Use of a dsRNA as claimed in Claim 1 wherein the dsRNA is a mismatched dsRNA.
3. Use of a dsRNA as claimed in Claim 2 wherein the dsRNA is a complex of a polyinosinate and a polycytidylate containing a proportion of uracil or guanine bases e.g. from 1 in 5 to 1 in 30 such bases.
4. Use of a dsRNA as claimed in Claim 3, wherein the dsRNA is of the general formula $rl_n \cdot (C_{12}U)_n$.
5. Use of a dsRNA as claimed in any preceding claim, wherein the composition comprises the dsRNA and an AIDS inhibition assisting agent.
6. Use of a dsRNA as claimed in Claim 5, wherein said assisting agent is a lymphokine.
7. Use of a dsRNA as claimed in Claim 6, wherein the lymphokine is an interferon or an interleukin.
8. The use of dsRNA as claimed in Claim 7, wherein the composition contains the dsRNA and an interferon in the ratio of 0.01 to 1000 micrograms of dsRNA to 0.1 to 100,000 IRU of interferon.
9. A method of rendering a biological fluid or cells resistant to infection with retrovirus or T-cell lymphotropic virus, e.g. AIDS virus, enhancing the resistance thereof, or mitigating the effect of infection therefrom comprising admixing or contacting said biological fluid or cells with an effective amount of a dsRNA as defined in any one of Claims 1 to 4.
10. A method as claimed in Claim 9 in which the biological fluid is human blood or a fraction thereof, or the cells are NK cells, T-cells, T-suppressor cells, T-helper cells, spermatozoa, ova or fetal cells.
11. A process of removing or inactivating retroviruses or T-cell lymphotropic viruses, e.g. AIDS viruses from a device for handling parenteral fluids or mitigating the effect of infection therefrom comprising contacting said device with a composition containing an effective amount of a dsRNA.
12. The use of dsRNA in the manufacture of a medicament for restoring an anergic immune state to a substantially normal immune state, as measured by testing the immune skin response.

Patentansprüche für die Vertragsstaaten: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Verwendung einer dsRNA zur Herstellung einer Zubereitung für eine Verwendung zur Behandlung von durch Retrovirus oder durch lymphotropen T-Zellen-Virus-induzierten Bedingungen, beispielsweise AIDS durch Therapie oder Prophylaxe.
2. Verwendung einer dsRNA nach Anspruch 1, wobei die dsRNA eine Fehlpaarungen aufweisende dsRNA ist.
3. Verwendung einer dsRNA nach Anspruch 2, wobei die dsRNA ein Komplex eines Polyinosinats und eines Polycytidylats ist, enthaltend einen Anteil an Uracil- oder Guandidinbasen, beispielsweise von 1 in 5 bis 1 in 30 derartiger Basen, ist.
4. Verwendung einer dsRNA gemäß Anspruch 3, wobei die dsRNA der allgemeinen Formel $rl_n \cdot (C_{12}U)_n$ entspricht.
5. Verwendung einer dsRNA nach einem der vorhergehenden Ansprüche, wobei die Zubereitung die dsRNA und ein AIDS-inhibierendes Hilfsmittel enthält.
6. Verwendung eines dsRNA nach Anspruch 5, wobei das Hilfsmittel ein Lymphokin ist.

EP 0 213 921 B1

7. Verwendung einer dsRNA nach Anspruch 1, wobei das Lymphokin ein Interferon oder ein Interleukin ist.

8. Verwendung einer dsRNA nach Anspruch 7, wobei die Zubereitung die dsRNA und ein Interferon in einem Verhältnis von 0,01 bis 1000 µg dsRNA zu 0,1 bis 100000 IRU Interferon enthält.

9. Verfahren zum Resistentsmachen eines biologischen Fluids oder Zellen gegenüber einer Infektion mit Retrovirus oder lymphotropen T-Zellenvirus, beispielsweise AIDS-Virus, zum Erhöhen der Widerstandsfähigkeit davon oder zum Abschwächen der Infektionswirkung davon durch Vermischen oder Kontaktieren des biologischen Fluids oder der Zellen mit einer wirksamen Menge einer dsRNA gemäß der Definition nach einem der Ansprüche 1 bis 4.

10. Verfahren nach Anspruch 9, wobei das biologische Fluid menschliches Blut oder eine Fraktion davon ist, oder die Zellen NK-Zellen, T-Zellen, T-Unterdrückungszellen, T-Helferzellen, Spermatozoen-, Ei- oder Fötuszellen sind.

11. Produkt aus menschlichem Blut oder einer Fraktion davon oder menschlichen Zellen und einer wirksamen Menge einer Fehlpaarungen aufweisenden dsRNA als Arzneimittel.

12. Verfahren zur Entfernung oder Inaktivierung von Retroviren oder lymphotropen T-Zellenviren, beispielsweise AIDS-Viren, aus einer Vorrichtung zur Handhabung von parenteralen Fluids oder zum Abschwächen der Wirkung einer darauf zurückgehenden Infektion durch Kontaktieren der Vorrichtung mit einer Zubereitung, die eine wirksame Menge dsRNA enthält.

13. Verwendung von dsRNA zur Herstellung eines Medikaments zur Wiederherstellung eines in wesentlichen normalen Immunzustands aus einem anergischen Immunzustand, gemessen durch Testen der Immunen Hautreaktion.

Patentansprüche für den Vertragsstaat: AT

1. Verwendung einer dsRNA zur Herstellung einer Zubereitung für eine Verwendung zur Behandlung von durch Retrovirus oder durch lymphotropen T-Zellen-Virus-induzierten Bedingungen, beispielsweise AIDS durch Therapie oder Prophylaxe.

2. Verwendung einer dsRNA nach Anspruch 1, wobei die dsRNA eine Fehlpaarungen aufweisende dsRNA ist.

3. Verwendung einer dsRNA nach Anspruch 2, wobei die dsRNA ein Komplex eines Polyinosinats und eines Polycytidylats ist, enthaltend einen Anteil an Uracil- oder Guandidinbasen, beispielsweise von 1 in 5 bis 1 in 30 derartiger Basen, ist.

4. Verwendung einer dsRNA gemäß Anspruch 3, wobei die dsRNA der allgemeinen Formel $r_n \cdot (C_{12}U)_n$ entspricht.

5. Verwendung einer dsRNA nach einem der vorhergehenden Ansprüche, wobei die Zubereitung die dsRNA und ein AIDS-inhibierendes Hilfsmittel enthält.

6. Verwendung einer dsRNA nach Anspruch 5, wobei das Hilfsmittel ein Lymphokin ist.

7. Verwendung einer dsRNA nach Anspruch 1, wobei das Lymphokin ein Interferon oder ein Interleukin ist.

8. Verwendung einer dsRNA nach Anspruch 7, wobei die Zubereitung die dsRNA und ein Interferon in einem Verhältnis von 0,01 bis 1000 µg dsRNA zu 0,1 bis 100000 IRU Interferon enthält.

9. Verfahren zum Resistentsmachen eines biologischen Fluids oder Zellen gegenüber einer Infektion mit Retrovirus oder lymphotropen T-Zellenvirus, beispielsweise AIDS-Virus, zum Erhöhen der Widerstandsfähigkeit davon oder zum Abschwächen der Infektionswirkung davon durch Vermischen oder Kontaktieren des biologischen Fluids oder der Zellen mit einer wirksamen Menge einer dsRNA gemäß der Definition nach einem der Ansprüche 1 bis 4.

10. Verfahren nach Anspruch 9, wobei das Biologische Fluid menschliches Blut oder eine Fraktion davon ist, oder die Zellen NK-Zellen, T-Zellen, T-Unterdrückungszellen, T-Helferzellen, Spermatozoen-, Ei- oder Fötuszellen sind.

11. Verfahren zur Entfernung oder Inaktivierung von Retroviren oder lymphotropen T-Zellenviren, beispielsweise AIDS-Viren, aus einer Vorrichtung zur Handhabung von parenteralen Fluids oder zum Abschwächen der Wirkung einer darauf zurückgehenden Infektion durch Kontaktieren der Vorrichtung mit einer Zubereitung, die eine wirksame Menge dsRNA enthält.

12. Verwendung von dsRNA zur Herstellung eines Medikaments zur Wiederherstellung eines im wesentlichen normalen Immunzustands aus einem anergischen Immunzustand, gemessen durch Testen der immunen Hautreaktion.

Revendications pour les Etats contractants: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Utilisation d'un ARN double brin dans la préparation d'une composition utilisable dans le traitement thérapeutique ou prophylactique d'états induits par des rétrovirus ou des virus lymphotropes de lymphocytes T, par exemple le SIDA.

2. Utilisation d'un ARN double brin selon la revendication 1, dans laquelle l'ARN double brin est un ARN double brin désapparié.

3. Utilisation d'un ARN double brin selon la revendication 2, dans laquelle l'ARN double brin est un

EP 0 213 921 B1

complexe d'un polyinosinate et d'un polycytidylate contenant une proportion de bases uracile ou guanidine, par exemple de 1 pour 5 à 1 pour 30 de telles bases.

4. Utilisation d'un ARN double brin selon la revendication 3, dans laquelle l'ARN double brin répond à la formule générale $rI_n \cdot (C_{12}U)_n$.

5. Utilisation d'un ARN double brin selon l'une quelconque des revendications 1 à 4, dans laquelle la composition comprend l'ARN double brin et un agent auxiliaire d'inhibition du SIDA.

6. Utilisation d'un ARN double brin selon la revendication 5, dans laquelle ledit agent auxiliaire est une lymphokine.

7. Utilisation d'un ARN double brin selon la revendication 6, dans laquelle la lymphokine est un interféron ou une interleukine.

8. Utilisation d'un ARN double brin selon la revendication 7, dans laquelle la composition contient l'ARN double brin et un interféron dans le rapport de 0,01 à 1000 µg d'ARN double brin à 0,1 à 100 000 IRU d'interféron.

9. Procédé pour rendre un liquide biologique ou des cellules résistants à l'infection par rétrovirus ou par virus lymphotropes de lymphocytes T, par exemple par le virus du SIDA, en exaltant leur résistance ou en atténuant les effets de l'infection, consistant à mélanger ou à mettre en contact ledit liquide biologique ou lesdites cellules avec une quantité efficace d'un ARN double brin tel que défini dans l'une quelconque des revendications 1 à 4.

10. Procédé selon la revendication 9, dans lequel le liquide biologique est du sang humain ou une fraction de celui-ci, ou les cellules sont des cellules NK (Natural killer), des lymphocytes T, des cellules T suppressives, des cellules T auxiliaires, des spermatozoïdes, des ovules ou des cellules fœtales.

11. Produit comprenant du sang humain ou une fraction de celui-ci ou des cellules humaines et une quantité efficace d'un ARN double brin désapparié, en tant que médicament.

12. Procédé d'élimination ou d'inactivation de rétrovirus ou de virus lymphotropes de lymphocytes T, par exemple de virus du SIDA, dans un dispositif servant à la manipulation de fluides parentéraux ou d'atténuation des effets de l'infection due à celui-ci, consistant à mettre ledit dispositif en contact avec une composition contenant une quantité efficace d'un ARN double brin.

13. Utilisation d'ARN double brin dans la confection d'un médicament pour rétablir, à partir d'un état immuno-energique, un état d'immunité sensiblement normal, mesuré par examen de la réaction immunitaire de la peau.

Revendications pour l'Etat contractant: AT

1. Utilisation d'un ARN double brin dans la préparation d'une composition utilisable dans le traitement thérapeutique ou prophylactique d'états induits par des rétrovirus ou des virus lymphotropes de lymphocytes T, par exemple le SIDA.

2. Utilisation d'un ARN double brin selon la revendication 1, dans laquelle l'ARN double brin est un ARN double brin désapparié.

3. Utilisation d'un ARN double brin selon la revendication 2, dans laquelle l'ARN double brin est un complexe d'un polyinosinate et d'un polycytidylate contenant une proportion de bases uracile ou guanidine, par exemple de 1 pour 5 à 1 pour 30 de telles bases.

4. Utilisation d'un ARN double brin selon la revendication 3, dans laquelle l'ARN double brin répond à la formule générale $rI_n \cdot (C_{12}U)_n$.

5. Utilisation d'un ARN double brin selon l'une quelconque des revendications 1 à 4, dans laquelle la composition comprend l'ARN double brin et un agent auxiliaire d'inhibition du SIDA.

6. Utilisation d'un ARN double brin selon la revendication 5, dans laquelle ledit agent auxiliaire est une lymphokine.

7. Utilisation d'un ARN double brin selon la revendication 6, dans laquelle la lymphokine est un interféron ou une interleukine.

8. Utilisation d'un ARN double brin selon la revendication 7, dans laquelle la composition contient l'ARN double brin et un interféron dans le rapport de 0,01 à 1000 µg d'ARN double brin à 0,1 à 100 000 IRU d'interféron.

9. Procédé pour rendre un liquide biologique ou des cellules résistants à l'infection par rétrovirus ou par virus lymphotropes de lymphocytes T, par exemple par le virus du SIDA, en exaltant leur résistance ou en atténuant les effets de l'infection, consistant à mélanger ou à mettre en contact ledit liquide biologique ou lesdites cellules avec une quantité efficace d'un ARN double brin tel que défini dans l'une quelconque des revendications 1 à 4.

10. Procédé selon la revendication 9, dans lequel le liquide biologique est du sang humain ou une fraction de celui-ci, ou les cellules sont des cellules NK (Natural killer), des lymphocytes T, des cellules T suppressives, des cellules T auxiliaires, des spermatozoïdes, des ovules ou des cellules fœtales.

11. Procédé d'élimination ou d'inactivation de rétrovirus ou de virus lymphotropes de lymphocytes T, par exemple de virus du SIDA, dans un dispositif servant à la manipulation de fluides parentéraux ou d'atténuation de l'infection due à celui-ci, consistant à mettre ledit dispositif en contact avec une composition contenant une quantité efficace d'un ARN double brin.

EP 0 213 921 B1

12. Utilisation d'ARN double brin dans la confection d'un médicament pour rétablir, à partir d'un état immuno-anergique, un état d'immunité sensiblement normal, mesuré par examen de la réaction immunitaire de la peau.

5

:

10

15

20

25

:

30

35

40

45

50

55

60

65

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☒ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.